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TEMPEH An Indonesian Fermented Soybean Food

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TEMPEH - AN INDONESIAN FERMENTED SOYBEAN FOOD

N. Iljas*, A. C. Peng, and W. A. Gould

The soybean has been hailed as a crop which could play a major role in feeding the world's starving people (40). The use of soybeans for food is especially appealing because of the high protein content (about 40%) which is the highest among legumes (120); the highest nutritive value among all proteins of plant origin (82); and the cheapest source of protein (18,84).

Processed soybean food has long been prepared in the Orient. A typical example is tempeh - a staple soybean food in the Indonesian daily diet. Tempeh has been studies extensively by both government agencies and universities (14,16,17, 21,22,33,34,36,41-52,66,70,71,73,78,81,92,94,98-105,111,112,114-119). Until now, however, no publication has comprehensively reported the results of these investigations. The purpose of this review is to compile published information on tempeh for those interested in fermented soybean foods.

PRODUCTION AND CONSUMPTION OF SOYBEANS

The world's soybean production was an estimated 43.4 million metric tons in 1971. This was 4.4% above the 1970 level and the seventh consecutive year of record production (113). The United States produced 74% of the world's soybean crop in both years and for the first time soybeans equaled corn production in cash receipts (25).

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About 40% of the world's soybean production is consumed as food and this is principally in the Orient (93). The rest is used for feed, fertilizer, and industrial purposes. In the Orient, Japan is probably representative with its soybean food products, such as tofu, shoyu, miso, monosodium glutamate, natto and kinako, and it is considered the most advanced country in soybean food technology (93).

NUTRITIVE VALUE

The soybean contains about 20% oil which is rich in essential fatty acids. Linoleic acid, an essential fatty acid, comprises more than 50% of the total oil contained in the edible portion of matured soybeans or soybean flour (120). Soybean oil, which is also rich in tocopherols and phosphatides, is considered a highly desirable vegetable oil in the human diet (84).

Mature soybeans and soybean flour contain little or no starch (38,55,126). The total carbohydrate content is about 34% (55,120) which is mainly sugars, with sucrose the largest component followed by stachyose, pentosans, galactans, and raffinose (38,55). The digestibility of soybean carbohydrate is intermediate (84) due to the presence of galactosido-sucrose (raffinose) which cannot be digested by humans because of the lack of alpha-galactosidase in the gastrointestinal tract (92).

Soybeans are also rich in phosphorus, iron, and magnesium, and contain a fair amount of calcium (84). They are a fair to good source of some of the B vitamins. The thiamine content is particularly high, with vitamin K also present in significant amount (84).

Soybeans are valued mainly as a rich source of high protein. Mendel and Fine in 1911 (68) were among the pioneers investigating the nutritive value of soybean protein. They observed that soybean protein could be utilized by men and dogs but not as well as that of a mixed diet containing meat and eggs. Five years later, Osborne and Mendel (80) reported that soybean meal supported normal growth of rats provided the meal has been adequately heated with water. They also observed that dry heating did not improve the nutritive value of the meal. Numerous reports have confirmed the improvement of the nutritive value of soybean products by cooking. The degree of the improvement is dependent on temperature, duration of heating, and moisture condition (62).

The superior protein quality of cooked or properly heated soybeans over that of uncooked is believed to be due to the inactivation of trypsin inhibitor which is present in the beans (88,89). However, the addition of methionine or cystine to unheated meal also improved protein utilization to essentially the same extent as proper heating (62). Interaction between soybean protein and minerals is probably also responsible for the lower nutritive value of the uncooked products (57).

Overheating, on the other hand, decreases the nutritive value of soybean protein. This decrease is due to the destruction of some amino acids, e.g. cystine, lysine, methionine, arginine, tryptophan, and serine (37,53,62,107,108), and is also due to the lower digestibility of protein (9), lower nitrogen absorbability (53), or lower availability of amino acids (74,89). Using defatted soybean flour, Taira _et al_. (109) found that the factors affecting the destruction of cystine, tryptophan, and serine were principally temperature and time of heating, while the amount of water was the factor primarily responsible for the breakdown of lysine and arginine. When a reducing sugar such as dextrose is present during heating, the decrease in nutritive value of protein becomes rapid (9). The amount of heat which will destroy sufficient cystine to make the amino acid first-limiting was found to be very close to the heat required to destroy growth inhibitors present in the raw beans (53).

Processing conditions also affect the nutritive value of soybean protein. Isoelectric protein, for example, has a different PER (Protein Efficiency Ratio) from that of calcium coagulated protein, and the same PER as freeze-dried and spray-dried samples of these proteins (19). Extracted soybean flour (low fat) has a different PER value from full-fat soybean flour (38).

Depending upon the processing conditions and the fraction of the soybean, the PER has been found ranging from 1.70 to 2.71 (19,35,38,117). Its protein score is found to be 70 (24,83) which is close to beef with a score of 74 (83) or 80 as calculated by another report (24). This level of quality is adequate for human diets according to Graham (32):

> "If the proteins in question have a score of 70 or better, if the PER in rats is in the vicinity of 2.0 if they can maintain adult humans in nitrogen balance, and if they will support 'normal' growth in children, they are almost certain to prove satisfactory as supplements to the diets of vulnerable groups".

DEFICIENCIES INHERENT IN SOYBEANS

Amino acid. Although the soybean is low in methionine (79), this can be improved or corrected by either complementing with other protein(s) or supplementing with the synthetic amino acid or both. Supplementation with sulfur-containing amino acids has encountered some difficulties, such as uniformity of mixing, government regulations, public antagonism, processing losses during preparation of food for consumption, etc. (18). Complementing seems to be the most practical method, and a number of high-protein foods designed by this technique are now available in various countries.

The soybean protein can be improved nutritionally by adding to it another plant protein which is high in sulfur-containing amino acids, such as sesame (*Sesamum indicum*) and ragimillet (*Eleusine coracana*). Alternatively the soybean protein may be added to another plant protein, such as wheat, which is low in lysine, while the soybean is high in this particular amino acid. Increasing the percent of soybean protein in relation to the white bread protein produces significant increases in protein quality to a maximum at 75% soybean protein to 25% white bread protein (121). Up to 6% soybean protein (which is equivalent to 10%

to 12% defatted soy flour or 14% to 16% full-fat soy flour) does not impair the overall acceptability of bread (69). The PER and biological value of white bread are increased when soybean flour is incorporated to an even greater extent than that of bread containing skim milk powder (54,110,113).

Beany Flavor. The biggest objection to the use of soybean as a food, especially by non-Orientals, is the so-called "beany" flavor or taste. This objectionable flavor has been called "raw bean flavor", "green bean-like" or "green bean flavor", "grassy", "nutty", "fishy", "painty", "hay-like", and "chalky".

A large number of volatile and non-volatile compounds which contribute directly or indirectly to the beany flavor have been found and identified. Fujimaki et al. (27) identified the volatile carbonyl compounds in defatted soybean flour as methanal, ethanal, n-hexanal, 2-propanone, 2-pentanone, 2-heptanone, 2-heptenal, and 2,4-decadienal, while those in the raw soybean were ethanal, n-hexanal, and 2-propanone. They also found four kinds of non-volatile carbonyl compounds, two of which seemed to be carbonyl ester and a carbonylic acid.

Sessa et al. (91) stated that the volatile carbonyl compounds contribute little to the overall soybean flavor. They identified acetone, n-hexanal, acetaldehyde, 2-heptenal, and a dicarbonyl compound as the major carbonyl compounds steam-distilled from raw full-fat soybean flakes, and n-hexanal, acetone, and acetaldehyde from defatted soybean flakes. They found that both kinds of soybean flakes had similar flavor characteristics in spite of differences in the number and kind of carbonyl constituents.

From defatted soybean flour, Arai et al. (5) identified at least seven phenolic acids with syringic acid as the main component. In another investigation on raw soybeans, Arai et al. (6) found nine compounds in the acidic fraction consisting of low molecular weight fatty acids with isocaproic and n-capric acids as the main components, and five amines in the basic fraction of soybean flavor components. In the investigation on raw soybean (ground), eight alcohols, one of them as an ester, were isolated (1) as volatile neutral components, with isopentanol, n-hexanol, and n-heptanol considered to be the important factors among this group which gave the bean-like odor to soybean. Arai et al. (4) also found some evidence concerning the interaction between soy protein and flavor components (n-hexanal and n-hexanol).

Mattick and Hand (67) isolated and identified ethyl vinyl ketone as a volatile component which develops in the soybeans and contributes to the raw bean odor and flavor. Wilkens and Lin (123) discovered 54 volatile components of whole-fat soybean milk, 41 of which were positively identified and 13 were tentatively identified. They also observed that hexanal was the major volatile component, but stated that the green-bean flavor of soybeans was probably ascribed to a mixture of many components.

Badenhop and Wilkens (8) noticed the production of 1-octen-3-ol in significant quantities during soaking of soybeans, and they presumed that it was formed by enzymatic action. From their investigation on the effect of processing methods on oxidative off-flavors of soybean milk, Wilkens et al. (124) concluded that the formation of the green bean-like flavor probably resulted from enzymatic action of lipoxygenase, since its appearance was rapid and occurred only in the unblanched product. Rackis et al. (87) examined the full-fat soybeans and found that when lipoxygenase was not inactivated, the oxidized lipids formed objectionable rancid flavors.

Studying the volatile flavor compounds in a reverted soybean oil, Smouse amd Chang (97) identified 71 compounds, 19 of which were acids and 39 non-acidic. The mechanism of formation of the compounds identified indicated that they were mostly primary or secondary autoxidation products of the hydroperoxides of the unsaturated fatty esters. The unpleasant soybean flavor substances of n-hexanal, n- hexanol, n-pentanol, and n-heptanol which were present in soybean tissues were also found in protein concentrate (2). The n-hexanal and n-pentanol were believed to be the main products resulting from lipoxygenase action on soybean oil (2).

Experiments have been performed in an attempt to mask, reduce, or if possible eliminate completely the offensive beany flavor. Realizing that the flavor was a possible result from enzymatic action, Wilkens et al. (124) used a high temperature to inactivate the enzyme, lipoxygenase, in the manufacture of soybean milk. An acceptable bland milk was obtained by grinding unsoaked, dehulled soybeans in water at a temperature between 80° and 100° C. (176° and 212° F.) and maintaining the mixture above 80° C. for 10 minutes (124).

Recent work with enzymes probably arose from the fact that fermentation changes the flavor of soybeans. Using papain, bromelin, pepsin, and nine other enzyme preparations of microbial origin, Fujimaki et al. (28) observed that the beany flavor of Promine-D, a commercial preparation of soybean protein isolate, was decreased in the early stages of digestion, while the bitter flavor and other unpleasant flavors increased with increasing digestion time. They also found that Molsin enzyme, a crude preparation of aspergillopeptidase, gave the best results.

Soybean curd and defatted soybean flour treated with aspergillopeptidase A preparation had less odor, while taste, color, and stability as measured by the TBA (thiobarbituric acid) reaction was superior to the product without enzyme treatment (75). Aspergillopeptidase and Molsin were effective in removing odorants, i.e. n-hexanal, n-hexanol, and n-heptanol from isolated soybean protein, but Molsin gave a less bitter proteolyzate (3). However, Aspergillus acid carboxypeptidase which was identical with the carboxypeptidase contained in Molsin did not seem to have a deodorant effect (3).

Sugimoto et al. (106) were successful in eliminating the beany flavor of isolated soybean protein used in preparing a beverage by treating it with acid-protease enzyme from Trametes sanguinea. Lemon flavor was then added.

Antinutritional Factors. Another defect of the soybean is the presence of antinutritional factors, namely trypsin inhibitors, hemagglutinins, saponins, and isoflavones (82,125). Booth et al. (12) indicated that the physiologically active constituents in raw soybean meal were a pancreatic stimulating factor, a trypsin inhibitor, a goitrogenic substance, saponins, estrogenic substances, hemagglutinin, and an anticlotting factor. Much research has been conducted to investigate the nature of those factors and their effects on the physiology of warm-blooded animals like rats, chickens and mice.

The soybean is known to contain a mixture of proteins, and 90% of the extractable proteins are globulins consisting of four fractions, i.e. 2S, 7S, 11S and 15S (72,126,127,130,132). Trypsin inhibitors are contained in the 2S fraction and the molecular weight ranges between 8,000 and 21,500 (126-129,131). The trypsin inhibitor was first discovered in 1944 (13). A year later a crystallized form was obtained and was called Kunitz' inhibitor (58,125). Kunitz' inhibitor, which is one of several trypsin inhibitors, is a single polypetide containing 194 amino acids with aspartic acid at the amino terminal and leucine at the carboxyl terminal (20,125,134). With the same terminal amino acid residues, Koide et al. (56) found 181 amino acids in the sequence of Kunitz' soybean trypsin inhibitor, but no molecular weight was reported in this study. Kunitz' inhibitor has a molecular weight of 21,500 and an isoelectric point at pH 4.5 (59). At this isoelectric point, it is insoluble in water but soluble in whey. Depending on the method of fractionation, five or more trypsin inhibitors have been isolated (76, 131) and Kunitz' inhibitor is one of them (77).

Ingestion of the trypsin inhibitor causes inhibition of the protein-digesting enzyme trypsin and suppression of growth, stimulation of the pancreas, and pancreatic hypertrophy (85,125). However, these disturbances seem to be interrelated. The inhibition of trypsin due to the inhibitor causes stimulation of the pancreas which results in pancreatic hypertrophy and excessive loss of critical amino acids contained in the pancreatic enzyme excreted in feces, and thus causes growth inhibition (12,85,125). Since the trypsin inhibitor is the protein known as a globulin, it is readily inactivated by moist heat and hence does not pose a serious problem in food (82,86,125).

Hemagglutinins are contained in the 7S fraction of the water-extractable soybean protein with a molecular weight of 111,000 (63,64,126-129,131). They are albumin in nature, soluble in water at the isoelectric point of pH 6.1, and contain mannose and glucosamine (63,64,125). Having multiple forms as do the trypsin inhibitors, at least four forms of hemagglutinins have been found (64,131). Hemagglutinin is known to cause clumping of red blood cells, but the agglutination of red blood cells in the body when eaten by animals or man is doubtful (125). Growth inhibition which may be accompanied by a decrease in food intake can occur due to hemagglutinin. However, this factor does not give any problem in foods because it is readily inactivated by moist heat (60) and by the enzyme pepsin which is present in the stomach (10,61,82,125).

Another antinutritional factor in the soybean is the saponin fraction which comprises about 0.6% of soybean meal (30). Saponins are non-proteinaceous and contain sugars, e.g. galactose, glucose, rhamnose, xylose, arabinose, and glucuronic acid (23,29,31), and they are heat stable (11). Although hemolysis of red blood cells is used for detecting soybean saponins, it does not occur when soybean meal is ingested, since the saponins pass through the stomach and the small intestine (31). Accordingly, soybean saponins do not create any problem in food, and can be considered as a non-antinutritional factor (125,131).

Isoflavones are another group of compounds in soybeans. Two forms have been found in the soybean, e.g. genistein and daidzein which are heat stable (125).

A third one, 6,7,4'-trihydroxyisoflavone, was isolated from tempeh (34), but its occurrence in soybeans is still undetermined. There has been no clearcut information about their antinutritional effects (125). Since they are only in relatively small amounts, 0.007-0.15% (125) they do not seem to present any problem.

TEMPEH

This is a popular Indonesian soybean food made from yellow soybeans by fermentation with a mold, Rhizopus sp. The fermentation eliminates the beany flavor of raw soybeans and gives the product a flavor which is bland but attractive. The preparation of this food is very simple and the fermentation is very rapid. Furthermore, this food is more digestible and more nutritious than plain cooked soybeans. Food poisoning has never occurred from consumption of this food and it is found to be highly acceptable even by Americans and Europeans (42).

The fermentation process of making tempeh produces desirable enzymes; destroys undesirable flavors, odors, and enzymes; adds flavor and odor; preserves; synthesizes desirable constituents such as vitamins; increases digestibility; changes the physical state; and produces color (48). In tempeh fermentation, undesirable flavors and odors of the raw substrate are destroyed or masked. The high digestibility of tempeh was noticed clearly when prisoners of war in southeast Asia during World War II were fed tempeh. Even those suffering from dysentery and nutritional edema were able to assimilate it (100,101).

Tempeh is a fermented soybean food characteristic of Indonesia and is not known in China or elsewhere (100). It is consumed as a main dish and meat substitute by many Indonesians who do not realize its nutritional quality. This product never becomes poisonous (111), and no food poisoning has ever occurred as a result of its ingestion (42), although Burkill (15) and Stahel (100) stated that longer fermentation makes it poisonous due to the production of ammonia. However, when coconut press cake is used as the substrate in place of soybeans, it may become poisonous due to _Pseudomonas_ infection (42,100).

Tempeh is also spelled tempe, and the word kedele or kedelee, meaning soybean, may be added to differentiate it from tempeh made from coconut press cake. Thus, tempeh kedele is made from soybeans and that from coconut press cake is called tempeh bongkrek (bongkrek = coconut press cake). Studies of tempeh have been made by investigators in Indonesia and Holland since about 1900. P.A. Boorsma was one of the pioneers as cited by Stahel (100) and Veen and Schaefer (111). In the U.S., studies on this product were started in 1960 at the Northern Marketing and Nutrition Research Division, Agricultural Research Service, U.S. Dept. of Agriculture, Peoria, Ill., and at the New York State Agricultural Experiment Station at Geneva.

The Tempeh Mold. The mold for the production of tempeh has been reported elsewhere in the literature (78,100,102,111,114) as _Rhizopus oryzae_ or as _Aspergillus oryzae_ (15). However, after observing at least 50 strains from various sources of tempeh, Hesseltine (41) concluded that the mold was _Rhizopus_ sp. NRRL 2710 with a more appropriate name _Rhizopus oligosporus_ Saito.

In the actual production of tempeh in Indonesia, a number of species and strains of mold are involved. Djien and Hesseltine (22) found that at least four species can be used, namely _R. stolonifer_, _R. oligosporus_, _R. oryzae_, and _R. arrhizus_. Hesseltine (42) stated that 40 strains of _Rhizopus_ can make acceptable soybean tempeh in pure culture. These strains are members of these six species: _R. oligosporus_ Saito, _R. stolonifer_ (Ehren) Vuill, _R. arrhizus_ Fischer, _R. oryzae_ Went & Geerligs, _R. formosaensis_ Nakazawa, and _R. achlamydosporus_ Takeda. Diokno-Palo and Palo (21) observed that tempeh obtained from fermentation by _Rhizopus_ sp. 12680 was better than that obtained by either _R. stolonifer_ NRRL 1477 or _Cunninghamella elegans_ A-12679.

R. oligosporus, the principal species used in Indonesia in the preparation of tempeh, is characterized by the sporangiophores which show no striations and which are very irregular in shape under any conditions of growth (42). The sporangiophores are short, unbranched, and arise opposite rhizoids which are very reduced in length and branching. This mold produces a large number of chlamydospores.

The tempeh fungus can use such materials as xylose, glucose, galactose, trehalose, cellobiose, sucrose, and stachyose, but not l-erythritol, lactose, or inulin (42,46,92). Sorenson and Hesseltine (98) found that stachyose, raffinose, and sucrose, which are the principal components of the soluble carbohydrates of soybeans, were not utilized as the sole sources of carbon, while common sugars such as glucose, fructose, galactose, and maltose as well as xylose and mannitol supported excellent growth, and arabinose, sorbose, sucrose, lactose, melibiose, raffinose, and stachyose were used poorly, if at all.

Hesseltine et al. (46) noticed that asparagine and ammonium sulfate were the best nitrogen sources. Sorenson and Hesseltine (98) demonstrated that ammonium salts and such amino acids as proline, glycine, aspartic acid, and leucine were excellent sources of nitrogen. Arginine was found to support significant growth without another carbon source, and sodium nitrate was not utilized as the sole source of nitrogen, while other amino acids were less suitable and tryptophan supported no growth at all (98).

Soybean oil is readily utilized by the tempeh mold because the mold is highly lipolytic (95,102). It produces a strong antioxidant (16,17,34,50,102), and possesses a strong capacity to decompose peroxides (95,96). The mold is also highly proteolytic which is important in breaking down the soybean protein (42,46,102,111,115), although it does not depend on any specific amino acid for growth as suggested by Sorenson and Hesseltine (98). Proteases appeared to be high in the strains of R. oligosporus (46). Later, two proteolytic enzyme systems were observed, one having an optimum pH of 3.0 and the other of 5.5. These enzymes were fairly stable in the pH range between 3.0 and 6.0 (115). Pectinase activity was hardly detectable (46).

In unbuffered substrates, this mold will produce enough ammonia to kill itself (102), but it does not produce a toxin (43). It forms only small amounts of amylase after a considerable time longer than the time required to make tempeh (46). The strains with good amylolytic activity are unsuited for fermentation of tempeh since they will break down starch to simple sugars which are then used to produce organic acids (43).

Stahel (100) observed that the fungus penetrated into seed lobes. In their microscopic observation, Veen and Schaefer (111) noticed that the convex side of the seed lobes of fermented beans was more readily perforated by the mycelium than was the flat (or inner) side, and the fungus seemed to penetrate only a few layers of cells. Steinkraus *et al*. (104) found the slight penetration of mycelia into underlying tissues of the beans and concluded that the digestion in the beans was mainly enzymatic. Steinkraus *et al*. (102) also observed that the individual cells of both cooked soybeans and tempeh were released from their intracellular matrix and became resistant to fracture when beaten in a Waring Blendor.

Methods of Preparation. Tempeh is made from yellow soybeans fermented with the tempeh mold *Rhizopus oligosporus* as the primary species. Different methods of manufacturing tempeh have been reported in the literature. Two methods were reported by Burkill (15). In the first one, the seeds are parboiled, left soaking in water for 2 or 3 days, and then drained. Some remaining water is removed by a slight pressing and a little heating, and the mush is spread upon frames in flat cakes, inoculated with tempeh fungus by addition of some previous preparations, then wrapped in banana leaves. After a day the mush is slightly stirred to ensure the even distribution of the hyphae and then left to ferment for another day. The second method is more elaborate and greater care must be exercised. The beans are washed and boiled (an operation taking two hours), transferred to cold water, and left under cover for 24 hours. Next the skins are removed, the kernels are boiled again and then steamed. The tempeh mold is prepared on a teak leaf by wrapping older tempeh and is allowed to dry for 2 days. The leaf is then emptied, cut fine, and sprinkled over the mush to inoculate. The inoculated mush is wrapped in banana leaves and left to ferment for 24 hours.

Stahel (100) mentioned a different native method of manufacture. The beans are boiled in about four volumes of water, cooled, and cleaned to remove the hulls. The dehulled, cleaned beans are soaked in water for 24 hours to ferment, and then boiled without changing the water. After boiling the water is discarded, the cooked beans are left standing to cool down, inoculated with well-made tempeh, and fermented in banana leaves.

Djien and Hesseltine (22) and Martinelli and Hesseltine (66) studied another different domestic method of manufacturing tempeh. The soybeans are soaked overnight so that the hulls can be easily removed and the next morning they are cleaned by hand. However, this is usually done by placing the soaked beans in a bamboo basket and treading on it by foot at the edge of a river or stream so that the seed coats, which are easily freed from the beans, float away with the running water. The cleaned, dehulled beans are then cooked in boiling water for about half an hour, spread on a bamboo tray ("tampah") or bamboo mat to cool and surface dry, inoculated with tempeh mold by mixing small pieces of tempeh from a previous fermentation (either fresh or dry), wrapped with banana leaves, and finally left to ferment at room temperature for 1 to 2 days.

Steinkraus et al. (103) reported two other domestic methods used in Indonesia - the long method and the short method. In the long method, the beans are soaked overnight in fresh water, washed, boiled in fresh water for 1 hour, and the skins are then removed by hand or foot. The dehulled beans are soaked again in fresh water (during this soaking a bacterial, acid fermentation is supposed to take place), and the water is discarded. The beans are then steamed for 90 minutes, cooled to about 37° C. (98.6° F.), inoculated with tempeh mold from a previous fermentation, wrapped in banana leaves, and left to ferment for 2 days. In the short method, the beans are heated in water for 1 hour and then soaked overnight. The loosened skins are removed or skimmed off and the beans are then steamed for 1½ hours, cooled, inoculated with tempeh mold, wrapped in banana leaves, and finally fermented.

The methods of manufacturing tempeh mentioned above are some of the common methods used in Indonesia which have been reported in the literature. Other methods may or may not have been reported. In the laboratory, two important methods have been adopted based on the domestic methods, but a pure mold culture is used instead of the mold from a previous fermentation.

One of these methods is that used by Hesseltine (41,46) and is similar to that used by Veen and Schaefer (111). The beans are washed and soaked in about three volumes of water overnight at room temperature. The next morning the seed coats are removed by hand by squeezing the beans between fingers or pressing them with the palm of the hand. During this action the beans are halved. The cleaned halved beans are cooked in boiling water for about 30 minutes, then drained and surface dried. After the cooked beans are cool (not higher than 40° C.), they are inoculated with a spore suspension of a pure culture of Rhizopus oligosporus grown on potato dextrose agar at 25° C. for 7 days. This suspension is obtained by washing one slant of mold culture with 1.5 ml. water and is sufficient for 100 g. of soybeans. The inoculated soybeans are then packed in petri dishes and fermented for about 20 hours at about 31° C., after which the beans are covered with white mycelia of the mold. The fresh tempeh with typical flavor is then ready. When larger amounts of tempeh are to be made, shallow wooden or metal trays with perforated bottoms and covers or perforated plastic bags or tubes should be used instead of petri dishes (66).

The other laboratory method is the one adopted by Steinkraus _et al_. (104). In this method the beans are soaked overnight in three volumes of water containing 10 ml. of 0.85% lactic acid per liter of water. After dehulling, the cleaned beans are returned to the acidified soak water and cooked at 100° C. for 90 minutes, drained, cooled to about 37° C., and inoculated with tempeh mold. The inoculum is prepared by inoculating the pure mold on a medium consisting of 100 g. of wheat bran moistened with 300 ml. of acidified soybean soak water sterilized in a Fernbach flask for 20 minutes at 120° C., incubated for 7 days at 37° C., lyophilized, and ground through a 20-mesh screen in a Wiley mill. One gram of inoculum is added per kilogram of cooked beans and mixed thoroughly. The inoculated beans are placed in perforated trays with perforated covers and incubated at 37° C. _Lactobacillus plantarum_, a lactic acid producing bacterium, can be added to the soak water in place of lactic acid. Acidification of the soak water to about pH 5.0 inhibits the growth of microorganisms which can cause spoilage.

The favorable temperature for growth of the tempeh mold is between 20° and 42° C. (22,46,103) and up to 45° C. (102), but the most desirable temperature is between 31° C. and 40° C. at which the fermentation of tempeh is completed in about 20 hours (22,46). However, Steinkraus _et al._ (103) illustrated that tempeh fermented at 37° C. for 36 hours was preferred by the majority of tasters, while Murata _et al_. (47) found that tempeh fermented for 48 hours at the same temperature (37° C.) was the most palatable.

As mentioned above, the cooked, inoculated beans are placed in covered containers in order to allow slow diffusion of air because the tempeh mold does not require an unrestricted exposure to air as many molds do. When there is too much air, the mold grows too fast and produces too much heat so that the temperature may rise to 49° C. and inhibit further growth (102). It has been observed that during fermentation the temperature increases from 37° to 45° C. and then falls again as the mold growth subsides (39). The soaking and cooking water has to be discarded because it contains a mold inhibitor which is heat stable and water soluble (44). Accordingly, fresh water is usually used and excess water is used in cooking.

Excellent tempeh can also be made from soybean grits (43,44,48), with the advantage of reduced soaking time to 2 hours. The disadvantage of using soybean grits is the high loss of solids in the soaking and cooking water (44). Difference in varieties of soybeans does not make as much difference in the tempeh as in ease of removing the seed coats (46). Removing the seed coats is essential as the mold cannot grow satisfactorily on the intact beans (103,104).

In studying the possibility of commercial production of tempeh, Steinkraus et al. (101,103) found two crucial problems, i.e. dehulling of soaked beans and developing a method for rupturing cells before spray-drying. Good tempeh can also be made from such cereals as wheat, oats, rye, barley, rice, and their combinations with or without soybeans (45,47,116,117), but poor growth of mold occurs when corn, sorghum or peanuts are used (47).

Changes in Chemical Composition. The preparation and fermentation of tempeh cause some changes in the chemical composition of the beans. Soaking, washing, dehulling, and cooking of the beans cause considerable loss of solids due to solution into the water. Fermentation further changes the composition. Steinkraus et al. (103) stated 1% to 2% solids lost due to soaking only, with the loss due to soaking and dehulling up to 5%. Stahel (100) reported 7% loss in the first boiling and 11% in subsequent processes. Soaking time is found to affect the amount of loss. Lo et al. (65) found losses of solids of 0.75%, 5.00%, and 9.75% for soaking times of 4, 24, and 72 hours, respectively. The solids leached out during the water treatment are primarily carbohydrates (92,100,111) and proteins (65), and some lipids (65) and minerals (90,111).

When dry dehulled soybeans are used for making tempeh, the loss of solids is higher up to 5% to 7% due to soaking and cooking (44).

Fermentation causes more loss of total solids, about 4% loss in dry weight (42). The total loss of solids due to soaking and fermentation becomes as high as 5% to 12% (90).

The high losses of solids consequently result in a low yield which has been reported to be about 72% (100,104), although a yield of almost 85% has also been investigated (51,52). The seed coats account for 9% to 10% solids removed from whole soybeans (100,103).

Fermentation of soybeans by the tempeh mold also causes an increase in soluble solids from 13.0% in cooked beans to 27.5% in tempeh (104). This change in soluble solids explains the higher digestibility of tempeh compared to plain cooked soybeans as stated by Veen and Schaefer (111). During fermentation, the pH gradually increases from 5.0 to 7.5 due to ammonia production in the later stages of fermentation (39,42,104). Fresh, properly fermented tempeh has been reported to have a pH value of 7.25 (51,52).

(a) Proteins and Amino Acids. Washing, soaking, dehulling, and cooking cause some losses in the protein of soybeans. Raw beans contain 42.99% protein on dry basis (65). After 24 hours of soaking, the protein content of the beans becomes 37.77% and after 72 hours, 26.59%. These data clearly show that the loss increases with the increase in soaking time. However, the temperature of the soak water does not affect the protein content (122).

The total nitrogen of the cooked beans has been found to remain about the same throughout fermentation, i.e. about 7.5%, but the soluble nitrogen increases from 0.5% to 2.0% (42,104). Veen and Schaefer (111) found a decrease in nitrogen content of the beans from 3.72% in raw soybeans to 3.16% in cooked beans and 2.67% in tempeh. Boorsma in 1900, as cited by Stahel (100) and Veen and

Schaefer (111), reported the loss of 2% of total protein due to fermentation. On the other hand, Wang et al. (117) and Murata et al. (70) noticed that the protein content of tempeh was slightly higher than that of the unfermented control.

In another experiment Murata et al. (71) observed that tempeh ordered from Indonesia contained 3.6% more protein than the control and the sample which was considered over-fermented contained 7.3% more protein than the control. Their samples prepared in Japan contained 1.8%, 1.6%, and 5.5% more protein than the control after fermentation for 24, 28, and 72 hours, respectively. Other reports (51,52) gave the figure of 42.44% protein contained in tempeh and 33.42% in raw, dehulled beans.

The amino acid composition of soybeans apparently is not significantly changed by fermentation (71,94,117). The increase or decrease is not more than 5% to 10% but the amount of free amino acid increases progressively during a 48-hour fermentation ranging from 1 for arginine to 85 times for alanine (71). A different result was found by Stillings and Hackler (105), who noticed the general decline of total amino acids due to fermentation, while free amino acids and amonia increased. Lysine and methionine have been found to decrease during the course of long fermentation (42,103).

(b) Lipids. Soaking also caused changes in lipid content of soybeans. Soybeans which have been soaked for 24 hours contain 23.4% fat (in terms of dry basis of the raw product) and those soaked for 72 hours contain 12.5% fat, while the non-soaked soybeans contain 24.0% fat (65). The temperature of soak water seems to have an effect on the fat content (122). Fermentation by the mold causes a decrease in the lipid content of soybeans (70,71,111,117). A decrease from 0.8% to 2.8% has been found by Murata et al. (70,71) and Wang et al. (117). Iljas (51) and Iljas et al. (52) stated that the lipid content in tempeh was about half of that in raw, dehulled soybeans, i.e. 11.68% in tempeh compared to 21.10% in raw soybeans.

Hesseltine (42) and Wagenknecht et al (114) stated that the total fat (ether extractable) in the beans remained relatively constant throughout fermentation, although about one-third of neutral fat was hydrolyzed into fatty acids by the tempeh mold. Such fatty acids are palmitic, stearic, oleic, linoleic, and linolenic acids, with linoleic acid predominant (42). Linolenic acid is the only fatty acid utilized by the mold, with about 40% of this fatty acid used (42). The production of fatty acids consequently results in an increase in the acid value of the lipid in the beans (71,114). Wagenknecht et al. (114) found the acid number for 69-hour fermented tempeh was 78.3, while that of cooked beans was 1.7.

The fact that tempeh is less prone to form peroxides and to become rancid indicates the presence of an antioxidant which is produced during fermentation (16, 17,33,102.103). Three antioxidants were later isolated and identified by Gyorgy et al. (34) as Factor 2 (or 6,7,4'-trihydroxyisoflavone), Daidzein (7,4'-dihydroxyisoflavone), and Genistein (5,7,4'-trihydroxyisoflavone). About 20 mg. of Factor 2 were isolated from 5 kg. of dry tempeh but the other two antioxidants were present in much smaller amounts (34,125). They presumed that these antioxidants act through their protection and preservation of the biologically, highly effective vitamin E in soybeans and by direct biological action. The activity of tempeh antioxidant was found to increase with fermentation time (50). The strong antioxidant activity can be judged from the peroxide value of tempeh of only up to 1.1 as compared to 18.3 to 201.9 in control soybeans (51,52,102,103). Murata et al. (70) found that the peroxide value of stored tempeh was only one-tenth that of stored soybean powder, and Ohta et al. (78) found a value considerably lower than that.

(c) Carbohydrates. It was mentioned previously that the carbohydrates are primary compenent of solids lost in the water during washing, soaking, dehulling, and cooking (92,100,111). Stahel (100) stated that 7% of the dry matter was lost at the first boiling and 11% more after 1 day of leaching and subsequent boiling, and this lost dry matter was mostly carbohydrates. Veen and Schaefer (111) also found a large quantity of carbohydrates lost due to soaking and cooking. No starch or dextrin and only 0.9% soluble carbohydrates were present in cooked beans, while there was a trace of starch, 2.5% dextrin, and 7.8% soluble carbohydrates in raw beans. Shallenberger et al. (92) found that the carbohydrate content of cleaned, cooked beans was less than half of that of raw beans, and this loss was largely at the expense of sucrose.

Reducing substances of the beans are decreased by fermentation (42,92,104). Melibiose, a reducing disaccharide, reached a maximum of 0.5% after 35 hours of fermentation, but then disappeared after 60 hours (92). On the other hand, sucrose, stachyose, and raffinose apparently can not be used or are used slowly by the tempeh mold (42). During a 72-hour fermentation, sucrose decreased slightly, stachyose decreased about twice as rapidly as sucrose and was nearly absent after 72 hours, while raffinose was in a steady concentration (92).

Hemicellulose decreases due to fermentation (42,111). The hemicellulose content (as glucose) of 2.8% in raw beans became 2.0% as the beans were cleaned and cooked, and after fermentation the hemicellulose content was only 1.1% (111). Fiber content, on the other hand, increases due to the development of mycelia of the mold (71,104,111). Murata et al (71) found an increase from 3.2% fiber in the control to 3.3% to 4.3% in tempeh. Steinkraus et al (104) illustrated an increase from 3.70% in dehulled beans to 5.85% in tempeh. Different results were reported by Wang et al (117), who found a decrease in fiber content from 3.9% in control beans to 3.1% in tempeh. They also presented a contradictory result on the carbohydrate content, which increased from 18.1% in control beans to 20.9% in tempeh.

(d) Vitamins. Like other components of soybeans, vitamins also undergo changes due to fermentation. Six vitamins have been reported in tempeh, i.e. thiamine, riboflavin, niacin, pantothenate, B-6, and B-12. Although the vitamins are not present in significant amounts (21), their presence in tempeh is worth mentioning when this product is to be used as an inexpensive but nutritious food. Thiamine decreases during fermentation (42,90,103), and about one-third of this vitamin present in cooked cotyledons is used by the fungus (90). Murata *et al.* (71) observed that thiamine content was slightly altered. It tended to increase at the beginning and then decreased during further fermentation. Riboflavin, niacin, vitamin B-6, and B-12 are increased (42,71,90,103). Riboflavin is about 3 to 5 times higher and niacin 3.4 times higher (90). Pantothenate was found to decrease by Steinkraus *et al.* (103), but Murata *et al.* (71) found it increased.

The amounts of vitamins present in soybeans and tempeh as found by Steinkraus *et al.* (103) are presented in Table 1. No major differences in B vitamins between normal impure tempeh fermentation and pure culture fermentation were reported by Roelofsen and Thalens (90).

Table 1.--A Comparison of Certain Vitamins in Soybeans and Tempeh (Concentration per Gram Sample).

Vitamin	Soybeans	Tempeh
Riboflavin	3 ug	7 ug
Pantothenate	4.6 ug	3.3 ug
Thiamine	10 ug	4 ug
Niacin	9 ug	60 ug
B-12	0.15 mug	5 mug

(e) Minerals. Changes of individual minerals in soybeans during fermentation have not been reported. However, the literature reports that the ash content of tempeh is not much different from that of unfermented beans. Murata *et al.* (71) found that the ash content of tempeh was 2.7% to 3.0% while that of unfermented control was 3.0%. Wang *et al.* (117) found even less decrease from 3.4% in control beans to 3.3% in tempeh. Roelofsen and Thalens (90) observed a decrease of about 0.2% to 0.4% ash. The decrease in ash content may have resulted from the loss of solids during soaking and cooking (90,111). Boorsma, as cited by Veen and Schaefer (111), observed that the ash content seemed to increase during fermentation. In another report, Murata et al. (70) presented the ash content of tempeh (lyophilized) as higher than that of control soybeans, i.e. 2.4% in tempeh and 2.0% in soybeans.

Nutritive Value. Jansen and Donath in 1924, as cited by Veen and Schaefer (111), proved in animal tests that tempeh protein was of excellent quality and supplemented the protein of rice well. This result is very meaningful to Indonesians since tempeh is used as a meat substitute by many of them, while rice is their basic food. Veen and Schaefer (111) found that tempeh was much more digestible than cooked beans and also much softer due to the decrease in hemicellulose. Much of the bean was solubilized by fermentation; e.g., more than half of the protein was broken down to water soluble products like amino acids. They also stated that less nutrients were lost during the preparation when compared to soybean curd.

Comparing tempeh with other Oriental soybean foods, such as tofu, natto, edamane, soybean sprouts, soybean milk, and soybean curd, and also with soybean protein isolate, concentrate, and flour, tempeh is the highest in PER, i.e. 2.48 (131). Gyorgy (33) found that rats fed tempeh showed better growth and greater resistance of blood cells to in vitro dialuric acid-induced haemolysis than rats fed plain boiled soybeans. At 10% protein intake, tempeh was superior to control soybeans in nutritive value, and the weight gain and PER were equal to skim milk at 10% and 20% protein intake. But, he stated further, when tempeh was hot-air-dried at 65.5° C. (150° F.), the PER was the same as unfermented soybean flour.

Hackler et al. (36), on the other hand, did not find the improvement in nutritive value and digestibility of soybeans fermented by the tempeh mold, but they stated that properly prepared tempeh contained high quality protein. Murata et al. (70) and Wang et al. (117) also found insignificant increase in PER of soybeans after fermentation. Murata et al. (71) stated that the increase in PER reported by Gyorgy (33) might be attributed to better availability of amino acids liberated from the beans during fermentation and to better digestibility of tempeh due to increase in soluble solids and nitrogen as reported by Steinkraus et al. (102,103,104).

Smith et al. (94) presented opposite results. They found that rats fed tempeh showed a small reduction in growth and PER compared with autoclaved and dehulled full-fat soybean meal. However, PER of tempeh was improved with methionine supplementation. The weight gain of rats on a diet of tempeh supplemented with 0.3% methionine was 203.2 g. compared to 160.0 g. on plain tempeh. The weight gain of rats on 14% casein was 165.0 g. and on full-fat soybean meal was 167.2 g. The Protein Score of tempeh was found to be equal to that of full-fat soybean meal, i.e. 63, while the Protein Score of autoclaved cotyledons was 65. Pancreatic hypertrophy did not occur in rats fed tempeh because the heat used in normal preparation of tempeh was sufficient to destroy the factors in raw soybeans responsible for poor growth and pancreatic hypertrophy. They indicated further that the loss of solids and proteins due to preparation before fermentation did not reduce the nutritive value of either cotyledons or full-fat grits (chips) used to make tempeh.

Although different results have been presented by workers mentioned above, one should not deny the success of tempeh as food for prisoners of war in southeast Asia during World War II as stated by Veen and Schaefer (111). As mentioned previously, fermentation causes the increase in vitamins, soluble solids and nitrogen, and the softness of the beans, and also causes the liberation of linoleic acid, an essential fatty acid. All of these indicate the better quality of tempeh compared to plain cooked soybeans.

Preservation. Tempeh is a perishable product and has a very attractive flavor. Its disadvantages make it necessary to consume the product shortly after fermentation is completed, and are the main reasons why it is manufactured daily on a small scale and sold fresh in Indonesia. Antioxidants which are present in tempeh prevent it from becoming rancid, but fermentation continues and ammonia is produced, giving an unpleasant flavor and odor.

Stahel (100) observed that tempeh deteriorated and became poisonous after 2 1/2 days in a package of banana leaves, but when the package was opened to let the product dry slowly by exposing it to the air, it was still edible even 2 days later. Thus, stopping the mold growth and further fermentation without losing the attractive flavor is an important factor when commercialization of tempeh is considered (93).

Fried tempeh in tin containers has been sold in the market in Indonesia (22). However, this method causes some loss of flavor (46). When tempeh is stored in a deep freezer, it deteriorates in appearance and is not as good as freshly made tempeh if it is cooked (46). A promising method was found by Hesseltine *et al.* (46), who sliced it at the end of fermentation. This sliced tempeh was cooked in boiling water for 5 minutes or until all mold and enzymes were destroyed and then frozen in a deep freezer. After 100 days of storage, the tempeh was thawed and deep-fried. The appearance, odor, and taste were almost the same as for tempeh cooked immediately after fermentation.

Canning and hot-air-drying at 60° C. of the sliced, 5-minute cooked tempeh are possible ways of preserving this food (51,52). The acceptability scores of samples of canned and dehydrated products after being deep-fried did not show significant differences from those frozen samples, although the canned samples were a little less acceptable even after 10 weeks. Hot-air-drying, however, causes reduction in soluble solids and nitrogen and reducing substances as shown in Table 2. Lyophilization causes less reduction in soluble nitrogen or reducing substances but not soluble solids (104). Nevertheless, preservation by deep-freezing, canning, and drying of cooked, sliced tempeh is possible from the flavor acceptability point of view.

Table 2.--Comparative Effect of Freeze-Drying vs. Hot-Air-Drying (69° C.) on Several Analyses of Tempeh.*

Tempeh Samples	% Reducing Substances	% Soluble Solids	% Soluble Nitrogen
Fresh	0.71	17.6	2.31
Lyophilized	0.41	19.5	1.19
Hot-Air-Dried	0.28	13.8	0.61

* Steinkraus _et al_. (104)

Acceptance and Potential Use. The soybean tempeh has a bland but attractive flavor and is highly acceptable to Americans and Europeans (42,111). The bland flavor makes it possible to add other flavoring agents such as onion, garlic, or others (99). The preparation of this food is very simple because only a single substrate is needed, while the low cost of preparation and of the soybeans makes this product very inexpensive. The fermentation time of 24 hours or less is very short compared to other fermented soybean foods like shoyu which takes 18 to 24 months and hemanatto, 6 to 12 months. The mold for fermentation belongs to a group of fungi not known to produce toxin, and food poisoning has never happened (42,111).

Because of those advantages and because this food is more nutritious and more digestible than plain cooked soybeans, it is suggested for child feeding in developing countries and has been introduced in southern Rhodesia (7). Its production on a larger scale is possible (101), and freezing, drying, or canning as a method of preservation can be used (46,51,52). A variety of high protein foods from tempeh may appear soon in the market as stated in the winter 1971 issue of Foods of Tomorrow (99):

> "Of all fermented foods, tempeh with its high ratings in taste, nutritional benefits, and simple, low cost processing techniques, appears to be the most likely candidate for Americanization, may be one of the next to appear in the U.S. market place."

Production of tempeh from other cereals such as wheat, oats, rye, barley, rice, and their combinations with or without soybeans, using the same tempeh mold and the same method of preparation, is proven possible (45,47,116,117). Such tempehs or their products may enter the market in the future.

The presence of antioxidants in tempeh is another potential value. Packett et al. (81) found that corn oil containing 50% tempeh showed higher antioxidant potential than those containing 25% tempeh, 0.01% alpha-tocopherol, or 0.03% alpha-tocopherol. Since there is only 20 mg. pure Factor 2 in 5 kg. of dried tempeh, which is equal to 0.0004%, while the other antioxidants in this product are much lower (34), the activity of these antioxidants is very high. This also means that tempeh may be used to prevent or slow down development of rancidity of foods (16,17).

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